PB-242 138

AIR POLLUTION EXPOSURE AND IMMUNOGLOBULIN LEVELS

MELOY LABORATORIES, INCORPORATED

PREPARED FOR
NATIONAL ENVIRONMENTAL RESEARCH CENTER

MARCH 1974

DISTRIBUTED BY:

National Technical Information Service
U. S. DEPARTMENT OF COMMERCE

163048

PB242138

EPA-650/1-75-005

March 1974

Environmental Health Effects Research

Reproduced by
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. Department of Commerce
Springfield, VA. 22151

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	PB 242 138
EPA-650/1-75-005		

4. TITLE AND SUBTITLE
Air Pollution Exposure and Immunoglobulin Levels

5. REPORT DATE
March 1974

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Meloy Laboratories, Inc.
Biological Products Division
6715 Electronic Drive
Springfield, VA 22151

10. PROGRAM ELEMENT NO.
1AA005

11. CONTRACT/GRANT NO.
68-02-0586

12. SPONSORING AGENCY NAME AND ADDRESS
Environmental Protection Agency
Research Triangle Park, NC 27711

13. TYPE OF REPORT AND PERIOD COVERED
Final - 8/23/72 to 6/30/73

14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

16. ABSTRACT

The objective of the study was to determine whether air pollution affects serum levels of immunoglobulins to a different degree in smokers than in nonsmokers. The sera tested were obtained from army recruits in the Chicago area, 517 smokers and 147 nonsmokers. The smokers and nonsmokers were further categorized by geographic location and by the presence of cough and phlegm. Quantitative determination of levels of IgA, IgG, IgM, and C3 in the samples of sera were by the method of single radial immunodiffusion described by Fahey and McKelvey. The results indicated that no significant differences existed between the mean concentrations of immunoglobulins when smokers were compared to nonsmokers by geographic location, nor were there significant differences between smokers with cough and phlegm and those without.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Air Pollution Blood serum	Smoking Immunoglobulin Cough Phlegm	06F

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Unlimited	Unclassified	20
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	3.25

EPA Form 2220-1 (9-73)

NOTES SUBJECT TO CHANGE

EPA-650/1-75-005

AIR POLLUTION EXPOSURE AND IMMUNOGLOBULIN LEVELS

by

Meloy Laboratories, Inc.
Biological Products Division
6715 Electronic Drive
Springfield, Va. 22151

Contract No. 68-02-0586
Program Element No. 1AA005

EPA Project Officer: Dr. Carl Hayes

Human Studies Laboratory
National Environmental Research Center
Research Triangle Park, N. C. 27711

Prepared for

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
WASHINGTON, D. C. 20460

March 1974

12

EPA REVIEW NOTICE

This report has been reviewed by the National Environmental Research Center - Research Triangle Park, Office of Research and Development, EPA, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into series. These broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and maximum interface in related fields. These series are:

1. ENVIRONMENTAL HEALTH EFFECTS RESEARCH
2. ENVIRONMENTAL PROTECTION TECHNOLOGY
3. ECOLOGICAL RESEARCH
4. ENVIRONMENTAL MONITORING
5. SOCIOECONOMIC ENVIRONMENTAL STUDIES
6. SCIENTIFIC AND TECHNICAL ASSESSMENT REPORTS
9. MISCELLANEOUS

This report has been assigned to the ENVIRONMENTAL HEALTH EFFECTS RESEARCH series. This series describes projects and studies relating to the tolerances of man for unhealthful substances or conditions. This work is generally assessed from a medical viewpoint, including physiological or psychological studies. In addition to toxicology and other medical specialities, study areas include biomedical instrumentation and health research techniques utilizing animals - but always with intended application to human health measures.

This document is available to the public for sale through the National Technical Information Service, Springfield, Virginia 22161.

Publication No. EPA-650/1-75-005

TABLE OF CONTENTS

	Page
Objective	1
Scope	1
Experimental Design	2
Methods and Materials	3
Results	8
Conclusions	15

1. Objective

The objective of this effort was to determine whether or not air pollution affects the serum levels of IgA, IgG, IgM and C3 to a different degree in smokers than in nonsmokers. The null hypothesis that there is no significant difference in the levels of these proteins in the serum was used as the basis of the study.

To test the null hypothesis, it was necessary to determine the serum labels of the four proteins in question by the method of single radial immunodiffusion described by Fahey and McKelvey[1] and to analyze the resulting data statistically. The serum tested were obtained from 147 nonsmokers and 517 smokers. These two categories were further broken into geographic area and the presence of cough and phlegm.

2. Work Scope

The task consisted of two parts: (1) the quantitative determination of the levels of IgA, IgG, IgM and C3 in 800 samples of sera (517 smokers, 147 nonsmokers and 136 unkeyed); and (2) analysis of data. The quantitative determinations were performed in a manner that most assured the technical correctness and unbiasedness of the results.

The analysis of the data was performed using standard statistical techniques utilizing a digital computer for the computations because of the large number of samples involved.

1. Fahey, J.L., and McKelvey, E.M., J. Immunol., 94:84-90 (1965).

3. Experimental Design

Originally, the Environmental Protection Agency was to supply the 800 serum samples necessary for the task. The initial experimental design and analysis were based on the fact that there would be 400 smokers and 400 nonsmokers. The actual samples received were not as evenly distributed as originally intended. In fact, Meloy Laboratories was supplied with 855 samples. These 855 samples had thirteen character code numbers. The key to this code was supplied by the EPA, but was incomplete. In addition, of the 855 samples, only 664 appeared on the key list. The 664 were divided into groups by a key category number ranging from 1 through 9 and distributed as follows:

Code	Area	Characteristics	Number Supplied
	Chicago	Smokers; cough and phlegm	23
2	Suburbs	Smokers; cough and phlegm	131
3	Rural	Smokers; cough and phlegm	126
4	Chicago	Smokers; no cough or phlegm	66
5	Suburbs	Smokers; no cough or phlegm	92
6	Rural	Smokers; no cough or phlegm	79
7	Chicago	Nonsmokers	38
8	Suburbs	Nonsmokers	88
9	Rural	Nonsmokers	21

Of these 664 samples, only 22.13% were nonsmokers Because of this deviation from the original distirbution, the analysis of data had to be reorganized (Section 4). The quantitative analysis of the 664 samples and 136 unkeyed samples was performed using the method originally proposed.

4. Methods and Materials

The study utilized quantitative immunodiffusion plates and the techniques described by Fahey and McKelvey[1]. To insure the consistency from plate to plate, one hundred 24-determination plates of each protein type were acquired and reserved for the study[2].

The samples were randomly divided into 15 groups of 51 samples and one group of 35 samples. One group of samples was run per day. A daily analysis consisted of setting up five plates of each protein type yielding a total of twenty plates per day.

On one plate of each protein type each day, four reference standards, a control serum, and seven samples were run in duplicate. The configuration of the other four plates consisted of the control serum and 11 samples in duplicate per plate. The sample locations were randomized using a random number generator to eliminate biased readings.

The plates were incubated at room temperature for 18 hours and resulting diameters determined using a micro-comparator. The diameters were read to the nearest 0.1mm.

The standards for IgG, IgA and IgM used for this study were calibrated against purified proteins and against the World Health Organization International Reference Preparation 67/95. The results of these determinations are expressed in milligrams per milliliter and International Units, (I.U).

1. Fahey, J.L., and McKelvey, E.M., J. Immunol., 94:84-90 (1965).
2. Meloy Laboratories, Biological Products Division, 6715 Electronic Drive, Springfield, Virginia 22151.

In the case of C3, there is no W.H.O. standard available, and the C3 level of the standard was established by calibration against purified proteins only.

The control serum that was utilized in the study was a well characterized pool of normal human serum obtained from normal donors and calibrated against the secondary standard in more than 40 determinations.

Daily results were analyzed by regression techniques. The standard curve of each pro_ein type was subjected to linear regression analysis using a digital computer. The resulting equation was used to compute the concentrations of the control points and the samples.

The control points were compared for each of the five plates and more than a 10% difference in any plate resulted in retesting all samples on that plate. Duplicate values computed for the samples were compared also. Deviations of more than 10% between duplicate values were marked for retesting.

Results from the calculations, including the control points and the standard curves for protein type were stored on magnetic tape for reference during the final analysis at the completion of the study.

At the completion of the 16 days of quantitative analysis, the resulting 16 standard curves for each of the four proteins were used to compute the mean regression equation for each protein.

The concentrations of the sera from each group and for each protein were subjected to standard statistical computations for the mean, variance, standard deviation, standard error of the

mean, coefficient of variation, skewness, and kurtosis. In addition, the range of values was determined for each protein in each group.

The regression equation for each daily group and each protein was used to compute the mean standard curve for each protein. The 95% confidence for each mean curve was used to determine if entire daily groups were to be repeated. All curves fell within the confidence limits and no repeats were necessary.

The concentrations in mg/ml and International units (I.U.) for IgG, IgA and IgM and the concentration in mg/ml for C3 resulting from the daily analysis were used to compute the mean concentrations for the groups. These values were utilized in the t-test to establish the significance of the differences between smokers and nonsmokers. The t-test was set up between groups as:

$$t_{n-2} = \frac{\bar{X}_1 - \bar{X}_2}{s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

Where: n_1 and n_2 are the numbers in the groups being compared;

$\bar{X}_1$ and $\bar{X}_2$ are the mean concentrations being compared;

$$s = \sqrt{\frac{(n_1 - 1)\,\delta_1^2 + (n_2 - 1)\,\delta_2^2}{(n_1 + n_2 - 2)}}$$

TABLE 1

Group Standard Curve Coefficients
(Section 4.1)

The general equation is: log Y = A +b (diameter)

	IgG		IgA		IgM		C3	
	A	b	A	b	A	b	A	b
Group 1	-.433	+.286	-.423	+.316	-1.43	+.633	-1.08	+.511
Group 2	-.406	+.265	-.466	+.273	-1.51	+.619	-1.14	+.543
Group 3	-.493	+.286	-.551	+.362	-1.18	+.546	-1.00	+.484
Group 4	-.392	+.267	-.496	+.329	-1.21	+.579	-.977	+.484
Group 5	-.367	+.253	-.349	+.275	-.996	+.531	-.939	+.485
Group 6	-.372	+.249	-.383	+.262	-1.19	+.544	-1.05	+.502
Group 7	-.342	+.250	-.516	+.335	-1.00	+.467	-.989	+.488
Group 8	-.394	+.264	-.469	+.253	-.805	+.277	-.744	+.410
Group 9	-.418	+.277	-.374	+.268	-.827	+.288	-.868	+.465
Mean	-.392	+.266	-.448	+.297	-1.12	+.498	-.976	+.486
Confidence Limits								
Intercept (A)	-.321 to -.493		-.308 to -.585		-.64 to -1.609		-1.21 to -.742	
Slope (b)	.237 to .295		.219 to .375		.234 to .762		.558 to .414	

TABLE 2

Compilation of Daily Standard Curve Coefficients,

Mean Standard Curve and Confidence Limits

Day	IgG A	IgG b	IgA A	IgA b	IgM A	IgM b	C3 A	C3 b
1	-1.455	+.263	-1.955	+.256	-1.949	-.354	-2.419	+.407
2	-1.362	+.257	-1.569	+.218	-2.052	+.364	-2.522	+.406
3	-1.241	+.252	-1.703	+.228	-1.891	+.347	-2.602	+.406
4	-1.165	+.234	-1.911	+.248	-1.990	+.377	-2.156	+.353
5	-1.136	+.232	-1.865	+.235	-1.944	+.359	-2.258	+.367
6	-1.345	+.254	-1.877	+.247	-2.059	+.375	-2.218	+.374
7	-1.127	+.225	-1.812	+.237	-1.997	+.369	-2.113	+.332
8	-1.178	+.238	-1.794	+.246	-2.057	+.380	-2.398	+.395
9	-1.236	+.246	-1.704	+.230	-2.100	+.382	-2.293	+.369
10	-1.296	+.246	-1.700	+.233	-2.006	+.374	-2.395	+.379
11	-1.058	+.224	-2.043	+.270	-1.792	+.296	-1.960	+.280
12	-1.026	+.222	-2.004	+.267	-2.018	+.370	-2.286	+.368
13	-1.183	+.243	-2.113	+.277	-2.270	+.428	-2.366	+.394
14	-1.086	+.227	-.546	+.226	-1.891	+.357	-2.204	+.359
15	-1.172	+.244	-2.052	+.274	-1.804	+.340	-2.115	+.350
16	-1.138	+.233	-1.825	+.240	-1.814	+.332	-2.215	+.361

	IgG	IgA	IgM	C3
Mean Regression Coefficients	-1.209 + .241	-1.842 + .245	-1.985 + .356	-2.276 + .37
Confidence Limits				
Intercept (A)	-.935 to 1.482	-1.540 to -2.18	-1.621 to -2.3	-1.919 to -2.63
Slope (b)	.208 to .274	.202 to .286	.269 to .437	.28 to .426

5. Results

5.1 Immunochemical Evaluation of Samples

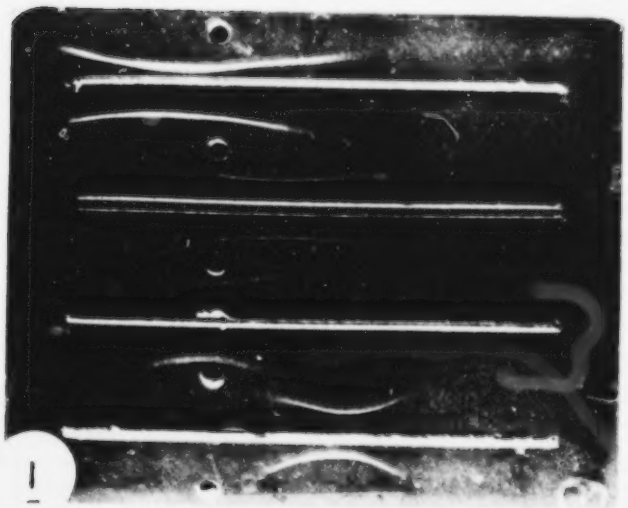
In order to establish the validity of the quantitative determination and the comparisons to be made from these determinations, it was necessary to establish that the proteins being measured in each of the sample groups were the same antigenically and that enzymatic breakdown of the proteins to be measured had not taken place during the four to five year storage period.

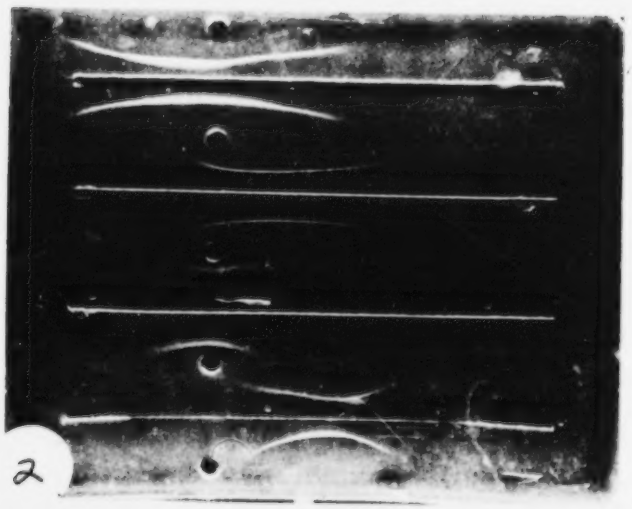
The degree to which the proteins were antigenically related between groups was established by the examination of the slopes, intercepts and comparison of the standard errors of the slopes in a parallel line assay. From each group, one serum was taken at random. Six two-fold dilutions in physiological saline were made of each of the nine sera selected, and the concentrations of each dilution for each protein were determined in duplicate using radial immunodiffusion. For each protein, the diameter was plotted against the logarithm (base 10) of the concentration for each dilution. The resulting 9 lines were compared by computing the 95% confidence limits and the mean regression equation. The results of the parallel line assays show that the antigens were the same in each of the groups.

The integrity of the proteins that were measured were evaluated on the undiluted nine samples used in the parallel line test. These samples were assessed by immunoelectrophoresis against appropriate specific antisera. Breakdown by any of the four proteins would have been readily determined by this method. There was no evidence that the integrity of any of the four proteins in any of the nine samples had been disturbed as shown in Figures 1-3.

FIGURE 1

Immunoelectrophoresis of Groups 1-3





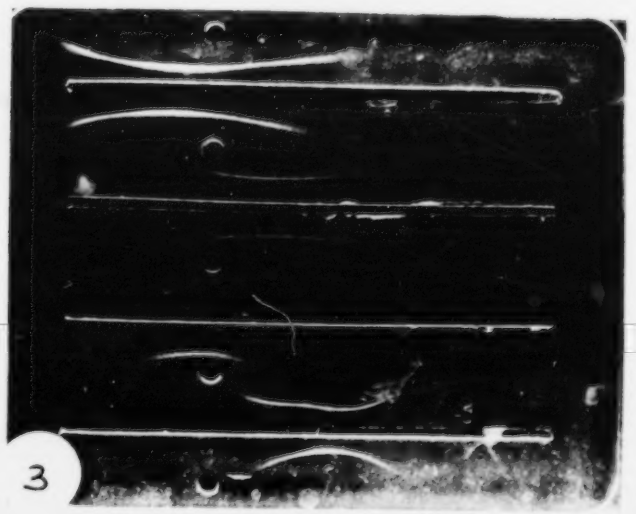
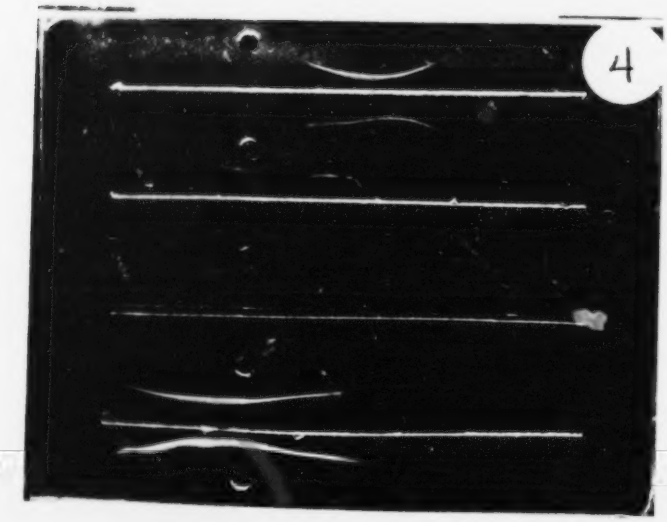


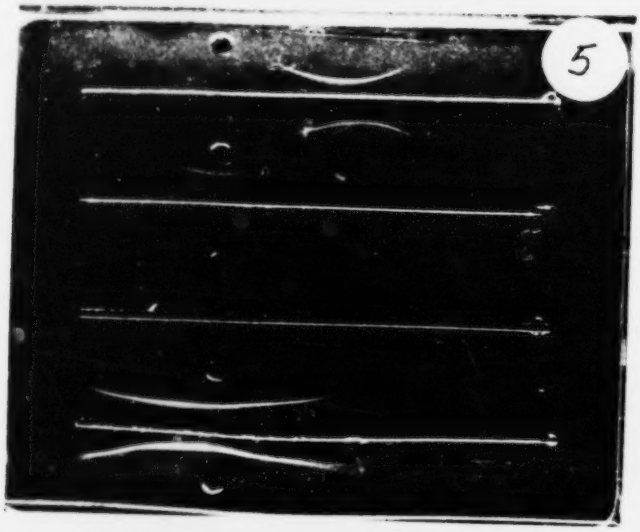
FIGURE 2

Immunoelectrophoresis of Groups 4-6



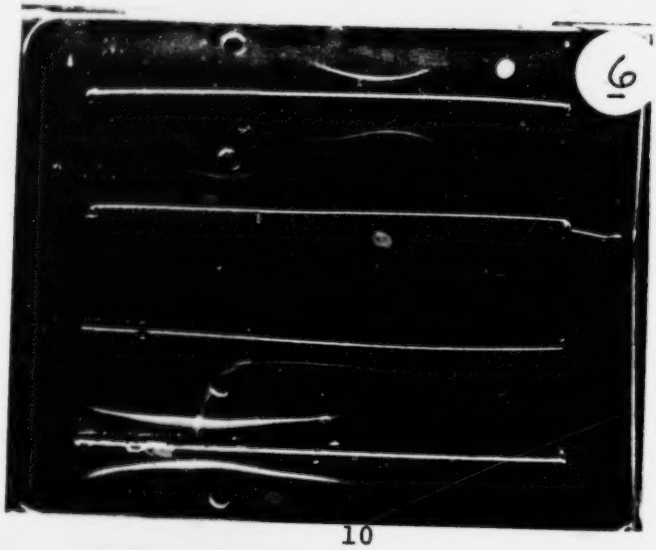
Control
Sample
Control
Sample
Control

Anti-IgG
Anti-IgA
Anti-IgM
Anti-C3



Control
Sample
Control
Sample
Control

Anti-IgG
Anti-IgA
Anti-IgM
Anti-C3

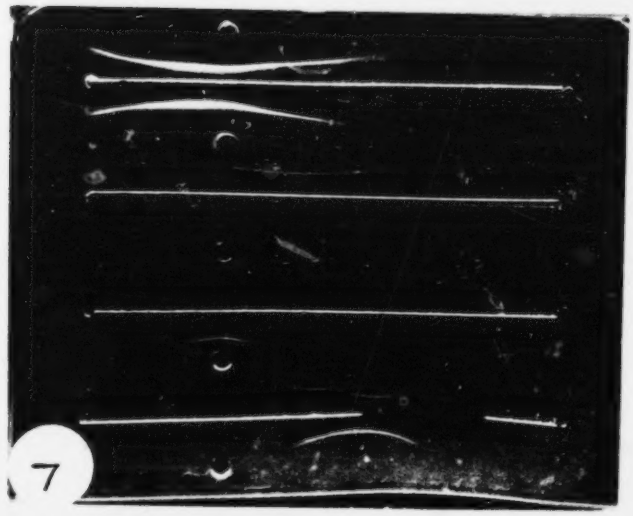


Control
Sample
Control
Sample
Control

Anti-IgG
Anti-IgA
Anti-IgM
Anti-C3

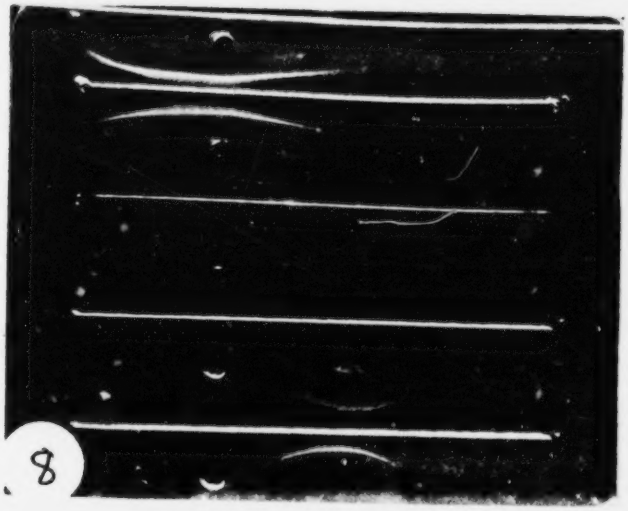
FIGURE 3

Immunoelectrophoresis of Groups 7-9



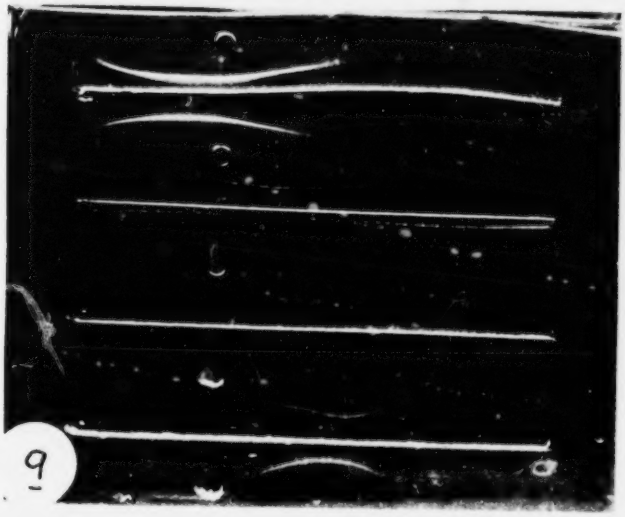
Sample
Control
Sample
Control
Control

Anti-IgG
Anti-IgA
Anti-IgM
Anti-C3



Sample
Control
Sample
Control
Control

Anti-IgG
Anti-IgA
Anti-IgM
Anti-C3



Sample
Control
Sample
Control

Anti-IgG
Anti-IgA
Anti-IgM
Anti-C3

5.2 Statistical Analysis of Groups

The data stored as daily results on magnetic tape were sorted into groups 1 through 9 using a computer. These groups were subjected to standard statistical computations which resulted in the range of values, mean concentration, standard error of the mean, variance, standard deviation, coefficient of variation, skewness and kurtosis for each group and each protein. These calculations were carried out for concentration both in milligram/milliliter and International Units/milliliter (Table 3).

In order to test the significance of the resulting differences in the mean concentration of each protein and each group, the t-test was formulated and applied to the groups, as discussed in section 4. The results of these inter-group comparisons are shown in Table 4. A t-value of less than 2.0 indicates that the differences in means are not significant. No group was shown to deviate significantly from any other group when this test was applied.

TABLE 3

Mean Concentrations of Proteins by Group

Group	n	IgG mg/ml	IgG IU/ml	IgA mg/ml	IgA IU/ml	IgM mg/ml	IgM IU/ml	C3 mg/ml
1	22	10.48	128	1.69	112	1.28	210	1.66
2	121	10.42	127	1.67	111	1.45	238	1.44
3	123	11.05	134	1.78	118	1.35	220	1.44
4	64	11.35	138	1.99	132	1.51	247	1.54
5	91	11.13	136	1.78	118	1.46	239	1.42
6	80	11.15	136	1.69	112	1.49	244	1.43
7	34	11.99	146	1.79	119	1.48	242	1.43
8	86	11.09	135	1.74	115	1.44	236	1.43
9	19	11.09	135	1.68	111	1.53	250	1.58

TABLE 4

Results of T-Test for Groups of Smokers vs. Non-Smokers

Group	IgG	IgA	IgM	C3
1 vs 7	.184	.041	.106	.148
2 vs 8	.036	.014	.002	.003
3 vs 9	.005	.022	.072	.089
4 vs 7	.038	.052	.010	.046
5 vs 8	.002	.008	.004	.004
6 vs 9	.004	.003	.016	.092

6. Conclusions

Normal levels of the three immunoglobulins, IgG, IgA and IgM, have been reported by Allansmith[3] as 7.1-15.4 mg/ml, 0.6-4.9 mg/ml, and .37-2.04 mg/ml respectively, and by Stiehm, et al[4] as 5.69-19.2 mg/ml, 0.61-3.3 mg/ml, and 0.47-1.47 mg/ml respectively. A comparison of the results from each of the 9 groups to these ranges certainly places them in the normal population.

In addition, the t-test establishes that no significant differences exist between the mean concentrations when smokers were compared to nonsmokers by geographic location. Additionally, inspection indicates no significant differences exist between smokers with cough and phlegm and those without.

Raw data required under this project was submitted in a Preliminary report.

[3] Allansmith, M. J. Pediat. 72:276 (1968).
[4] Stiehm, E.R., and Fudenberg, H.H. Pediat. 37:715 (1966)